[0027] FIG. **9** illustrates a two point calibration curve according to one embodiment of the present disclosure; and

[0028] FIG. **10** is a decision tree schematically illustrating a decision support algorithm according to one embodiment of the present disclosure.

DETAILED DESCRIPTION

[0029] Devices and methods for performing point of care diagnostic tests for detecting and quantifying at least one analyte in a biological sample (e.g., a body fluid). Disclosed herein are assay cassettes and testing devices that can be used to provide rapid, accurate, affordable laboratory-quality testing at the point of care. Such assay cassettes and testing devices are designed to provide rapid, quantitative test results in a point-of-care setting or the like where, in the past, only qualitative or semi-quantitative results have typically been available. Likewise, such assay cassettes and testing devices may eliminate or replace expensive, centralized clinical testing equipment and technical personnel. Such testing device may include automated data reporting and decision support.

[0030] In one embodiment, a diagnostic test system is disclosed. The system includes a lateral-flow chromatographic assay cassette and a testing device that includes data collection and data analysis capabilities. The testing device is configured to interface with and analyze output of the lateral-flow chromatographic assay cassette.

I. Diagnostic Test Systems

[0031] Referring to FIG. **1**, perspective view of a diagnostic test system **100** is illustrated. The diagnostic test system **100** includes a lateral-flow chromatographic assay cassette **105** and means for collecting assay data from the lateral-flow chromatographic assay cassette **105**.

[0032] The lateral-flow chromatographic assay cassette **105** includes a plastic housing **107** containing a test strip, which is generally a plastic strip laminated with porous material that permits lateral flow of liquid. The illustrated lateral-flow chromatographic immunoassay cassette **105** includes a sample application zone **110** and an analysis zone **130**.

[0033] When a sample **120** is applied to the lateral-flow chromatographic immunoassay cassette **105** at the sample application zone **110**, the sample **120** diffuses through the strip in flow direction **125** toward the analysis zone **130**. In the embodiment illustrated in FIG. **1**, the analysis zone **130** includes a test line **140** that includes at least one capture ligand selected for capturing at least one analyte of interest in the sample **120**. The analysis zone **130** further includes at least first and second calibration standard lines **150***a* and **150***b*. Additionally, the analysis zone may include a positive control line **170** that may be configured to provide an indication regarding whether or not sample has diffused though the strip and whether or not the assay is functioning. For example, the positive control line **170** may include a water soluble dye that is positioned and configures to indicate that the sample has flowed the length of/travered the test strip.

[0034] The analyte(s) of interest, the first and second calibration standards, and the positive control can be detected on their various target lines, **140**, **150***a*, **150***b* and **170**, respectively, with various reporters. The reporters **160** for each of the various target lines, **140**, **150***a*, **150***b* and **170**, may be the same or different. Examples of suitable reporters include, but are not limited to, visible and fluorescent dyes, latex beads, enzymes, gold nanoparticles, silver nanoparticles, titanium nanoparticles, europium fluorophores, quantum dots, and the like. Quantum dots are nano-scale materials that can produce excited emission at particular wavelengths depending on their size and shape. Quantum dots can be used in immunoassays where dyes have traditionally been used. However, quantum dots are generally superior to traditional organic dyes on several counts: quantum dots are typically much brighter that organic dyes (owing to their high extinction coefficients combined with a comparable quantum yield to fluorescent dyes) as well as their stability (i.e., much less photobleaching). For example, it has been estimated that quantum dots are 20 times brighter and 100 times more stable than traditional fluorescent reporters.

[0035] Emission from the various reporters can be excited by a number of sources. In the illustrated embodiment, an LED light source **180** is used illuminate the analysis zone **130** of the lateral flow assay cassette **105**. Illumination by the light source **180** may produce a detectable signal that includes at least one of emission (e.g., fluorescence), color, reflectance, diffuse scattering (i.e., scattering and absorbance), elastic light scattering, chemiluminescence, chemifluorescence, transmission, plasmon surface resonance, or absorbance from the reporters. A lens **190** (e.g., a collimating lens) and a detector **195** (e.g., a CCD or CMOS camera) are used to collect data from the reporters and the first and second calibration standards.

[0036] When the sample **120** is applied to the diffusion strip of the lateral-flow chromatographic assay cassette **105**, the liquid in the sample carries the analyte of interest through the diffusion strip in flow direction **125** into the analysis zone **130** where it can be captured by the capture ligand line **140**. The first and second calibration standard lines **150***a* and **150***b* are selected to provide a detectable signal that correlate to non-zero concentration values of the analyte of interest. For example, the first and second calibration standard lines **150***a* and **150***b* may include an amount of the analyte of interest or another material pre-bound to the diffusion strip of the lateral-flow chromatographic assay cassette **105**. The reporter **160** may be a diffusible material that can bind to the capture ligand line **150** and the first and second calibration standards **150***a* and **150***b* in an amount proportional to the amount of bound ligand is present in each line. In response to illumination by the light source, the reporter **160** bound to each of lines **140**, **150***a*, and **150***b* provides a signal that can be used to calculate a calibration curves and, in turn, determine the concentration of the analyte of interest in the sample **120**. A more detailed discussion of methods for deriving analyte concentration from the data of the first and second calibration standards **150***a* and **150***b* and the capture line **140** is discussed in greater detail elsewhere herein.

[0037] In one type of lateral-flow chromatographic immunoassay cassette, the test strip is divided into four domains, which can be made of only one kind of material or several kinds of material (e.g., up to four different kinds of materials). The first domain is for sample addition. It functions to remove viscous and particulate materials in the sample and also to condition the sample solution for the reactions in the following domains. The second domain is a mobile-phase with a color conjugate. In one embodiment, the color conjugate may be made from conjugation between a visible color marker (e.g., colored beads, colloidal gold, fluorescent dyes, etc.) and a detection antibody. The detection antibody can bind a specific antigen in the sample (e.g., an analyte of interest or a positive control substance) and forms an antigen-color con-